

Photoprotein as an internal light source for a photoelectrochemical cell employing a semiconducting oxide electrode

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Inorganic-bio nanocomposites combining photoproteins (aequorin, AEQ) with a Pt-doped α -Fe₂O₃ (Pt- α -Fe₂O₃) thin film could induce a photoelectrochemical reaction without an external light source. Blue emission originated from Ca²⁺ binding to AEQ excited the n-type semiconducting Pt- α -Fe₂O₃ under an anodic bias, then an anodic photocurrent was clearly observed in a basic solution.

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Key-words : Photoelectrochemical cell, Photoprotein, Bioluminescence, Oxide semiconductor, Bandgap excitation

[Received November 14, 2016; Accepted December 26, 2016; Published online February 24, 2017]

The present study substantiates that bioluminescence generated from photoproteins can induce photoelectrochemical reaction of inorganic semiconductors. Photoelectrochemical cells (PECs) using semiconductor electrodes have been explored in diverse purposes such as solar batteries,¹⁾ photoelectrolysis,²⁾ and photocatalysts.³⁾ In all PECs, photon energy irradiated to the semiconductors is transformed into chemical or electrical energy. In other words, the PECs cannot work without any light source. The present study proposes a new form of PECs that does not require any external light sources. As previous related researches, Tatsuma and coworkers have reported the photocatalysts with energy storage ability by combining two kinds of semiconductors.^{4,5)} Under light irradiation, photocarriers generated in one semiconductor are transferred and stored in another on the basis of difference in band positions between them. As a result, the photocarriers stored cause redox reactions even under dark condition. Matsumoto et al. have claimed remote photocatalytic oxidation of a solid substrate apart from an irradiated semiconductor photocatalyst.⁶⁾ In this communication, we design a novel PEC employing photoproteins as an internal light source. Since photoproteins emit light as a result of a bioaffinity reaction, binding of photoproteins to semiconductors can conduct photoelectrochemical reaction without any external light source. Here, we fabricated inorganic-bio nanocomposites composed of photoprotein (aequorin, AEQ) and Pt-doped α -Fe₂O₃ (Pt- α -Fe₂O₃) with a narrow band gap that can be excited by visible light. AEQ consists of apoprotein (apoaquorin) and a chromophore moiety (coelenterazine) bonded to an oxygen molecule. When a trace amount of Ca²⁺ is present, the coelenterazine changes a conformation along with a release of CO₂ and a blue emission.⁷⁾ Consequently, photoelectrochemical reaction of Pt- α -Fe₂O₃ can be initiated by the blue emission originated from AEQ. The accomplishment will lead to a development of an unprecedented photocatalytic reaction system with an internal light source.

A Pt- α -Fe₂O₃ thin film on a Pt foil was fabricated through a photoelectroless deposition technique and subsequent heat treatment on the basis of the procedure previously reported.⁸⁾ More concretely, the Pt foil was immersed in a mixed solution of 0.03 M FeSO₄, 0.03 M acetic acid and 1.5 mM H₂PtCl₆ (pH ~ 3), where the solution was thermostated at 313 K and saturated with O₂ gas throughout the deposition. In addition, the Pt foil was exposed to visible light from a 300 W Hg-Xe lamp by cutting off UV radiation with a long path filter (>400 nm). After the deposition for 20 h, the Pt foil covered with a transparent and pale yellow film was washed with deionized water and then was annealed at 1073 K in air for 1 h to obtain thermodynamically stable α -Fe₂O₃. At the initial stage of deposition, Fe²⁺ is oxidized to Fe³⁺ through the local cell mechanism, that is, Fe²⁺ releases an electron to the Pt foil, on the other hand, a dissolved O₂ molecule receives an electron from the Pt foil. In other words, the O₂ behaves as an oxidizer for Fe²⁺. Since the oxidized iron species (Fe³⁺) have low solubility at the pH, the ions are easily hydrolyzed, producing oxyhydroxide (FeOOH). The visible light irradiation to the Pt foil induces further growth of FeOOH because the preformed FeOOH is an n-type semiconductor and holes produced in the valence band continuously oxidizes Fe²⁺. The as-synthesized FeOOH has a tunnel structure composed of several FeO₆ octahedrons and anions such as SO₄²⁻ and PtCl₆²⁻ occupy inside the tunnels.⁹⁾ As a result of the post annealing, the thin film was crystallized as thermodynamically stable hematite (α -Fe₂O₃) accompanied by Pt⁴⁺-doping into the hematite lattice. Judging from comparable ionic radius of Pt⁴⁺ (VI coordination: 62.5 pm) with Fe³⁺ (64.5 pm),¹⁰⁾ the substitution of Pt⁴⁺ ions for Fe³⁺ sites seems to be feasible. The doping of Pt⁴⁺ with higher positive valence is expected to bring about a rise in carrier density of an n-type α -Fe₂O₃ semiconductor.

Figure 1(a) displays a surface SEM image of the Pt- α -Fe₂O₃ thin film. The film consists of numerous nanowires with diameters of 10–100 nm. Such microstructure with a high surface area may be useful for adsorption of a large amount of protein. According to literatures, an isoelectric point of α -Fe₂O₃ in an aqueous solution is located at ca. pH = 8,¹¹⁾ while AEQ has a

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[‡] Preface for this article: [DOI http://doi.org/10.2109/jcersj2.125.P4-1](https://doi.org/10.2109/jcersj2.125.P4-1)

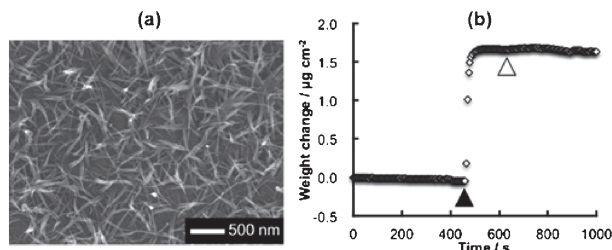


Fig. 1. (a) Surface SEM image of a Pt- α -Fe $_2$ O $_3$ thin film prepared by photoelectroless deposition and subsequent heat treatment. (b) Variation in the weight of a Pt- α -Fe $_2$ O $_3$ thin film on a Pt foil during adsorbing of AEQ estimated from QCM. Firstly, an acetate buffer solution (ABS, pH = 4) was circulated to the surface of the Pt- α -Fe $_2$ O $_3$ thin film, then the AEQ dissolved in the ABS (0.1 mg/mL) was introduced at the time shown as the close triangle. Finally, a tris-HCl buffer solution (0.1 M, pH = 9) flowed at the open triangle in order to confirm a tight binding between the Pt- α -Fe $_2$ O $_3$ thin film and AEQ.

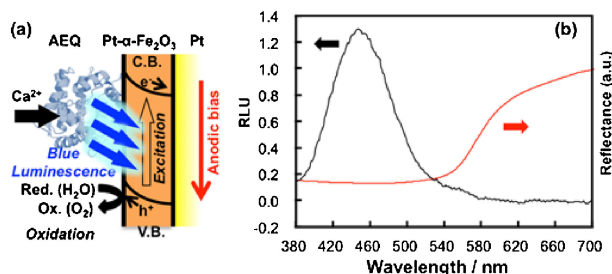


Fig. 2. (a) Schematic illustration of photoelectrochemical reaction of an AEQ-adsorbed Pt- α -Fe $_2$ O $_3$ thin film under anodic polarization. (b) Emission spectrum (black) of AEQ at pH = 9 combined with a reflectance spectrum (red) of a Pt- α -Fe $_2$ O $_3$ thin film measured using an integral sphere inner-coated with BaSO $_4$. The luminescence reaction of AEQ was initiated by addition of 0.1 M Ca(NO $_3$) $_2$ (0.1 mL), then the spectrum was measured at a scan rate of 5000 nm/min.

negative surface charge over a wide pH range (pH = 2–10).¹² Therefore, AEQ dissolved into an acetate buffer solution at pH = 4 can be adsorbed on the Pt- α -Fe $_2$ O $_3$ through an electrostatic interaction.

The Pt- α -Fe $_2$ O $_3$ thin film assembled in a flow type reactor was exposed to an AEQ solution at room temperature. The weight change of the film was monitored by the quartz crystal microbalance (QCM) method using a thin film fabricated on a Pt-coated quartz sensor. Figure 1(b) shows variation in surface weight of sensor covered with the Pt- α -Fe $_2$ O $_3$ thin film. The rapid weight gain was observed immediately after the introduction of the AEQ solution, and the gain was maintained even after exposure to a basic buffer solution (pH = 9) that might charge both negatively. This fact suggests AEQ was tightly bound to the surface of Pt- α -Fe $_2$ O $_3$ nanowires. Consequently, it was confirmed that nanocomposites consisting of AEQ and Pt- α -Fe $_2$ O $_3$ (denoted below as AEQ-Pt- α -Fe $_2$ O $_3$) could be fabricated by means of the simple adsorption.

Figure 2(a) illustrates a driving mechanism for the proposed photoelectrochemical cell using AEQ-Pt- α -Fe $_2$ O $_3$. An AEQ molecule catches a Ca $^{2+}$ selectively, resulting in blue light emission. Under anodic polarization, the emission of AEQ excites Pt- α -Fe $_2$ O $_3$ that can absorb visible light because the emission spectrum of AEQ is largely overlapped with an absorption region of Pt- α -Fe $_2$ O $_3$ as shown in Fig. 2(b). The fact that AEQ adheres to Pt- α -Fe $_2$ O $_3$, implying the blue light is effectively absorbed in Pt- α -Fe $_2$ O $_3$. The photogenerated electrons in the conduction band

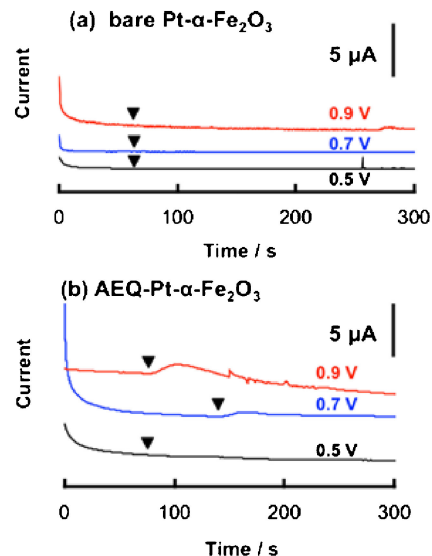


Fig. 3. Transient currents of Pt- α -Fe $_2$ O $_3$ (a) without or (b) with adsorbing AEQ under several anodic biases when 10 mM Ca(NO $_3$) $_2$ was introduced at the points denoted as the closed triangles. The addition of Ca(NO $_3$) $_2$ was performed just after reaching constant current.

(C.B.) will migrate to an external circuit under the anodic polarization, while the holes in the valence band (V.B.) will oxidize some chemical species in the solution. Taking into account the fact that the solutions circulated do not include any solutes to be oxidized, the holes will be consumed by oxidation of H $_2$ O (Red.), that is, production of O $_2$ (Ox.). Therefore, an anodic photocurrent should be observed under the polarization. AEQ in a natural jerry fish is hybridized with green fluorescent proteins (GFP) and hence the blue emission of AEQ is absorbed by GFP, producing green fluorescence.¹³ On the other hand, the present PEC transforms the blue emission into electric current.

The actual electrochemical measurements were performed in a flow type electrochemical cell. The SUS tube to introduce solutions and Ag | AgCl (3 M NaCl) inserted into the flow system were used as a counter and reference electrode, respectively. As emission intensity of AEQ was gradually increased with an increase in a solution pH examined (7 < pH < 9),¹⁴ the photoelectrochemical measurements were carried out at pH = 9 to activate AEQ strongly. Figure 3 shows the transient current responses under anodic polarizations of Pt foils covered with (a) the bare Pt- α -Fe $_2$ O $_3$ and (b) the AEQ-Pt- α -Fe $_2$ O $_3$ thin films when a Ca $^{2+}$ solution was introduced at 298 K.

A 0.1 M tris-HCl buffer solution at pH = 9 flown in advance was changed to a 10 mM Ca(NO $_3$) $_2$ (pH = 9) at the points denoted as the closed triangles. It is well-known that n-type semiconductors form a Schottky barrier at interface with a solution under anodic bias above E_{FB} . On the other hand, irradiation of photon energy enough to excite the semiconductor causes generation of anodic photocurrent based on migration of photo-produced electrons to a current collector (Pt) and oxidation of chemical species in the solution by holes left in the valence band. In the cases of AEQ-free (bare) Pt- α -Fe $_2$ O $_3$ [Fig. 3(a)], due to a lack of the light source (AEQ), the current was not altered even after exposing to the Ca(NO $_3$) $_2$ solution. Needless to say, the exposure of a bare Pt electrode to Ca $^{2+}$ also had no effect on a current response. In contrast, as shown in Fig. 3(b), the anodic current across AEQ-Pt- α -Fe $_2$ O $_3$ clearly increased by contacting with Ca $^{2+}$, and the current jump got larger at more positive applied potentials. These experimental findings suggest that the

proposed photoelectrochemical reaction actually occurred during the introduction of Ca^{2+} . At present, due to irreversible flash emission of AEQ, the occurrence of anodic photocurrent was restricted to a short reaction period less than about 50 s, then the current returned to the base line. To regenerate AEQ bound to Ca^{2+} , treatment with ligands (EDTA, etc.) is demanded to remove the bound Ca^{2+} .¹⁵⁾ On the other hand, if enzymes catalyzing a chemical reaction with bioluminescence such as luciferase or peroxidase are used as internal light sources, sustainable generation of a photoanodic current will be realized.

The present study examined whether the photoelectrochemical cell consisting of AEQ (light source) and the Pt- α - Fe_2O_3 thin film (photoanode) was operatable without exposure to radiation from an external light source. The addition of Ca^{2+} to a solution exposing the AEQ-adsorbed Pt- α - Fe_2O_3 thin film under anodic polarization generated photoanodic current. This fact meant that the blue emission from AEQ interacting with Ca^{2+} excited Pt- α - Fe_2O_3 with a narrow band gap and higher carrier density compared with undoped α - Fe_2O_3 , which was confirmed by the Mott-Schottky plots. Even though the anodic photocurrent observed here was small less than $5 \mu\text{A}/\text{cm}^2$ at present probably due to weak emission of AEQ, the experimental findings could extend photoelectrochemical devices not requiring an external light source such as photocatalysts or photovoltaic cells. Hence, our further efforts will be devoted to develop a state-of-the-art photocatalytic reaction system composed of other photoproteins and oxide semiconductors.

Acknowledgements The present study was partly supported by JSPS KAKENHI Grant No. 26410244.

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